

REMARKS

Claims 1-18, 20-26, 29-36 and 38-46 are pending in the present application. Claims 1-18, 29-36 and 45-46 are withdrawn from consideration. Claims 20-26 and 38-44 are under consideration in the Final Action dated December 18, 2002. Claims 20-26 and 38-44 have been rejected under 35 U.S.C. §102(b) as allegedly anticipated by Thomson et al. Science 282:1145-1147 (1998). Claims 19-26 and 37-44 have also been rejected under 35 U.S.C. §102(e) as allegedly anticipated by Thomson, U.S. Patent No. 6,200,806.

This response addresses each of the Examiner's rejections. Accordingly, the pending claims are in condition for allowance or at least in better condition for appeal. Favorable consideration is respectfully requested.

Applicants observe that the Examiner's reasons are the same for both the rejection based on Thomson (1998) and the rejection based on U.S. Patent 6,200,806 to Thomson et al. Therefore, Applicants address both rejections together and refer to the two Thomson references as "Thomson et al."

Applicants previously submitted that the methods taught by Thomson et al. do not point to the importance of the feeder cells for the differentiation of ES cells. The Examiner contends, however, that Thomson et al. do teach the importance of feeder cells for the state of differentiation of human and primate cells in that Thomson et al. purportedly teach that, unlike mouse ES cells, feeder cells are required for maintaining the undifferentiated state of primate and human ES cells. Applicants also submitted that Thomson et al. only teach the spontaneous differentiation of ES cell, not the specific induction of differentiation. The Examiner contends, however, that Thomson et al. specifically teach the parameters that affect the differentiation of the cell lines are the feeder layer, the cell density, and various growth factors such as allowing

the cells to proliferate without LIF. The Examiner also contends that it is known that if the ES cells are allowed to grow at high density, differentiation of the ES cells will occur. Therefore, it is the Examiner's opinion that the present claims do not recite method steps which are different from those disclosed by Thomson et al. The Examiner acknowledges that the differentiation disclosed by Thomson et al., could be viewed as spontaneous to the extent that the specific factors, which induce the cells to differentiate, are not specifically known. However, it is the Examiner's opinion that neither the instant claims nor the present specification teach those unknown factors.

In response to the Examiner's contentions, Applicants respectfully submit that the present claims are directed to an *in vitro* method of inducing somatic differentiation of undifferentiated, pluripotent human embryonic stem cells by growing the stem cells under culture conditions that induce somatic differentiation.

As described in the specification on page 20, there are some fibroblast feeder layers which can induce differentiation in either the extra embryonic differentiation or somatic differentiation streams. On page 20, lines 23 to 25, the specification further teaches that somatic differentiation *in vitro* of the ES cell lines is a function of (i) the period of cultivation following subculture, (ii) the density of the culture and (iii) the fibroblast feeder cell layer. Specifically, the specification has provided the conditions that induce somatic differentiation, contrary to the Examiner's allegation. Such conditions include a combination of (i) feeders that could support proliferation and renewal of stem cells with (ii) prolonged cultivation and/or high density that did not kill the cells, but restricted their self renewal and directed them to differentiate towards somatic tissues. These conditions are presently recited in the claims.

Applicants respectfully submit that the Thompson et al. citations do not provide any insight that the type of fibroblast feeder layer can affect the outcome of the differentiation. Thomson et al. teach that feeder layers help maintain undifferentiated cells, but do not appreciate that various feeder layers can influence somatic or extra embryonic differentiation. In contrast, as stated on page 20, line 19 and page 21, line 22 of the specification, the method of preparation and handling of the mouse embryo fibroblasts, the mouse strain from which the fibroblasts are derived and the quality of the particular batch, may affect and may favour stem cell renewal, extraembryonic differentiation or somatic differentiation. Hence, the specification not only recognizes that the fibroblast feeder layers are important, but also recognizes that there are subsets of the types of fibroblast feeder layer which would support controlled differentiation of the embryonic stem cells. Once a batch of fibroblast feeder cells is identified, such batch can be stored and resurrected for subsequent use in directing the induction of somatic differentiation.

The specification further provides, at the bottom of page 21, that "the modulation of stem cell growth by appropriate use of the fibroblast feeder layer and manipulation of the culture conditions provides an example whereby somatic differentiation may be induced *in vitro* concomitant with the limitation of stem cell renewal without the induction of wide spread cell death or extraembryonic differentiation." This would then favor differentiation of somatic cells as described in the following paragraph on page 22.

Applicants submit that the present inventors uniquely recognized that the various fibroblast batches differed in their potential to produce somatic versus extraembryonic differentiation. See page 21, line 4 to 19 of the specification. The present inventors had to screen batches to find those that favored somatic differentiation. Examples of feeder cell lines which are extremely effective in inducing somatic differentiation include B-83, which is the

product of an SVJ-129 X SVJ-129 and is still in use today. On the other hand, feeder cell line B-72, which is a product of an inbred cross of SVJ-129 X SVJ-129, was marked as a throw out of fibroblasts because this particular cell line either killed the ES cells or drove the ES cells to extraembryonic differentiation.

Accordingly, Applicants respectfully submit that the specification adequately teaches screening fibroblast feeder cell lines for those that favor somatic differentiation and the use of a differentiation inducing fibroblast feeder layers (which favors somatic differentiation) to induce a differentiated somatic lineage or multiple differentiated somatic lineage under conditions that do not permit continued stem cell renewal but do not kill stem cells or induce their unidirectional differentiation into extraembryonic lineages.

In Thompson et al., while the cells spontaneously differentiated, there was no induction or purposeful direction of the embryonic stem cells toward a somatic differentiation. Applicants respectfully submit that cells must be cultured in specific conditions as disclosed in the specification and recited in the present claims, e.g., claim 20. There is no teaching or suggestion in Thompson et al. that these specific conditions must be employed to induce somatic differentiation of the embryonic stem cells.

In summary, Applicants submit that Thompson et al. do not teach culturing cells under conditions which do not induce extraembryonic differentiation and cell death and promote proliferation of undifferentiated stem cells, which conditions are taught in the present specification and recited in the claims. Thompson et al. also do not teach culturing cells on a particular type of differentiation inducing fibroblast feeder layer which favors somatic differentiation, because Thompson did not appreciate that certain subsets of fibroblast feeder layers did in fact exist and specific fibroblast feeder layers are required to induce somatic

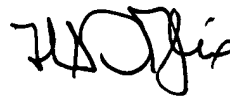
differentiation. Therefore, Applicants respectfully submit that the present specification teaches, and the instant claims recite, conditions that are not disclosed or even recognized in Thomson et al.

Furthermore, Applicants respectfully submit that the term "inducing" by definition means prevailing upon or bringing on a result by artificial means. In the context of the present invention and in light of the specification, the term is clearly understood by those skilled in the art to mean that a condition is employed to control the outcome of the differentiation. Specifically, the condition employed in the claimed methods is the use of differentiation inducing fibroblast feeder layers which favor somatic differentiation. In contrast, Applicants submit that Thompson et al. do not provide any enabling disclosure for methods of inducing somatic differentiation.

Accordingly, Applicants respectfully submit that the presently claimed methods are not taught by Thomson et al. Withdrawal of the rejection under §102(b) based on Thomson (1998) and the rejection under §102(e) based on the '806 patent is therefore respectfully requested.

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



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